FastRNA® Pro Soil-Indirect Kit

Rapid Isolation of Total RNA from Soil Supernatants Using the FastPrep® Instrument

Revision # 6075-050-4H01

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Application Manual

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Catalog # 6075-050

50 Samples

Storage temperature:
Ambient temperature (15–30°C)

DO NOT expose RNA*pro*™ Solution to light for extended periods of time.
Store in the original bottle in the closed kit box.

TABLE OF CONTENTS

1. Introduction to the FastRNA® Pro Soil-Indirect Kit and the FastPrep® Instrument

2. Kit Components and User Supplied Materials
2.1 FastRNA® Pro Soil-Indirect Kit Components
2.2 User Supplied Materials

3. Important Considerations Before Use
3.1 Preparation of RNAMATRIX® Wash Solution
3.2 Separation of Organic Materials from Soil
3.3 Preparing to Isolate Total RNA
3.4 Sample Lysis with the FastPrep® Instrument
3.5 RNA Purity, Humic Substance and Inhibitor Removal and Downstream Applications

4. Safety Precautions

5. Basic Protocol for All Soil Samples

6. Optional Centrifugation Through Quick-Clean Spin Filters

7. Example Data: Total RNA Isolation and RT-PCR

8. Troubleshooting
8.1 Lower Than Expected or No RNA Yields
8.2 Suspected RNA Degradation
8.3 Properties of the RNA Pellet
8.4 Genomic DNA Contamination
8.5 RT-PCR Inhibition
8.6 Mucopolysaccharide/Carbohydrate Contamination

9. Recommended Reference Format for Publication

10. References

11. Related Products
11.1 FastRNA® Pro Soil-Direct Kit (Cat# 6070-050)
11.2 Other Related Products

12. Product Use Limitation & Warranty

1. Introduction to the FastRNA® Pro Soil-Indirect Kit and the FastPrep® Instrument

The FastRNA® Pro Soil-Indirect Kit quickly and efficiently isolates total cellular RNA from microorganisms and other specimens found in soil. It is designed for use with the FastPrep® Instrument, a benchtop device that uses a proprietary vertical angular motion to homogenize samples by multidirectional, simultaneous impaction with lysing matrix particles (1). The FastPrep® Instrument provides an extremely quick, efficient and highly reproducible homogenization that surpasses traditional extraction methods using enzymatic digestion, sonication, blending, douncing and vortexing.

Soil biodiversity is directly affected by its physical and chemical composition and by environmental factors. Evidence indicates total soil biodiversity can be underestimated by approximately 90% when an in vitro culturing method is used to approximate the total number of organisms present (2, 3). For this reason, extraction of total RNA from soil has been used to detect specific genes from unculturable microorganisms, to provide a method to isolate and identify individual strains of interest, estimate soil biodiversity, estimate soil microorganism metabolic activity and to clone expressed genes (4, 5, 6, 7).

Nucleic acid extraction from soil can be performed using a direct or an indirect method. The indirect method utilizes an initial separation of microorganisms and other biological specimens from the soil followed by lysis of the organisms and RNA purification (8, 9). The indirect method also permits soil incubation with growth media to amplify living organisms prior to RNA isolation if accurate measurements of microbial diversity are not required. The direct method consists of extracting nucleic acid from microorganisms and other biological specimens directly from soil (10). FastRNA® Pro Soil Kits are available from Qbiogene for both direct (Cat # 6070-050) and indirect (#6075-050) RNA isolation methods.

Soil types differ in the type and amount of organic materials. The largest and most chemically significant fraction of natural organic matter is the humic substances, which include humic acid and fulvic acid (11). The amount and type of humic substances in a soil sample are established by a combination of environmental conditions, vegetation and topography, and will vary among soil types and even within soil at the same location. Humic substances frequently give soil a yellow/brown color and have been shown to inhibit Taq polymerase activity at concentrations as low as 0.1mg/ml (12, 13). The FastRNA® Pro Soil Kits purify RNA in a process that removes humic substances and other inhibitors, and efficiently inactivates cellular RNases during homogenization to prevent RNA degradation.

The FastRNA® Pro Soil-Indirect Kit offers two levels of RNA purification that permit tailoring the protocol to the soil sample and downstream applications. In the first level purification, RNA is purified from contaminating soil products by selective binding to RNAMATRIX®. For the vast majority of soil types, RNAMATRIX® purification will provide RNA that is colorless and free of RT-PCR inhibitors for use in downstream applications. In the event further processing is required, a second level of purification through Quick-Clean Spin Filters will provide additional purification of colorless and contaminant-free RNA.

2. Kit Components and User Supplied Materials

2.1 FastRNA® Pro Soil-Indirect Kit Components

RNA*pro*™ Solution	55 ml
Soil Filtration Cheesecloth	1 each
Inhibitor Removal Solution	12 ml
RNAMATRIX® Slurry	0.6 ml
RNAMATRIX® Binding Solution	35 ml
RNAMATRIX® Wash Solution Concentrate	15 ml
DEPC-Treated H_2O	30 ml
Lysing Matrix E	50 tubes
Quick-Clean Spin Filters	50 filters
Catch Tubes	50 tubes
User Manual	1 each
MSDS	1 each
Certificate of Analysis	1 each

2.2 User Supplied Materials

Resuspension Solution (either deionized H_2O, 10 mM Tris [pH 7.0 - 8.0], or 100 mM NaCl)
RNase Erase® (See Section 11, Related Products)
FastPrep® Instrument (See Section 11, Related Products)
100% Ethanol
Chloroform
Centrifuge capable of spinning at least 10 ml of liquid at less than 500 rpm (~20 x g)
Centrifuge capable of spinning at least 10 ml of liquid at 5000 rpm (~1,800 x g)
Microcentrifuge
Chilled 70% Ethanol (prepared with DEPC-treated H_2O)
Chilled Isopropanol
1.5 or 2.0 ml RNase-free Microcentrifuge Tubes
Agarose (See Section 11, Related Products)
Gel Loading Dye (See Section 11, Related Products)
Size Marker for Electrophoresis

3. Important Considerations Before Use

3.1 Preparation of RNAMATRIX® Wash Solution

The FastRNA® Pro Soil-Indirect Kit contains a bottle with 15 ml of RNAMATRIX® Wash Solution Concentrate. Before using this solution, add an equal volume (15 ml) of 100% ethanol and mark on the bottle label the date ethanol was added. Store at room temperature.

3.2 Separation of Organic Material from Soil

The FastRNA® Pro Soil-Indirect Kit protocol incorporates incubating the soil sample with Resuspension Solution to separate microorganisms and other biological specimens from the soil using low speed centrigation prior to lysis and RNA purification. The supernatant is then filtered through cheesecloth to remove remaining soil particles (NOTE: It is important NOT to substitute sterile gauze, Kim-Wipe™ tissue or commercially available coffee filters as significant RNA yield reduction will occur.) Following filtration, microorganisms and biological specimens are concentrated from the Resuspension Solution by a second centrifugation. Pelleted organic material is then lysed in RNA*pro*™ Solution by the FastPrep® Instrument.

3.3 Preparing to Isolate Total RNA

The presence or introduction of RNase during the procedure may result in sample degradation. It is strongly recommended that the user minimize the potential for RNase contamination by wearing gloves throughout the procedure, using DEPC-H_2O and by treating pipettmen, work area, gel box and gel comb with RNase Erase® (see section 2.2). Additional RNA handling methods and precautions are found in references 14 and 15.

Confirm the Lysing Matrix E tubes spin freely and will not scrape the microcentrifuge wall during centrifugation.

Add RNA*pro*™ Solution to the sample as soon as possible to initiate RNase inhibition. FastPrep® Instrument homogenized and non-homogenized samples are stable in RNA*pro*™ Solution for up to 24 hours at room temperature or 4ºC. It is best to process the soil sample through the complete protocol as soon as possible following collection.

3.4 Sample Lysis with the FastPrep® Instrument

The fill volume in the lysing matrix tube after the addition of RNA*pro*™ Solution to the sample should allow sufficient air space in the sample tube for efficient FastPrep® Instrument processing. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

Qbiogene's Lysing Matrix particles and tubes have been rigorously tested and validated in the FastPrep® Instrument. The use of non-Qbiogene products with the FastPrep® Instrument is not recommended and may result in sample loss or instrument failure.

A single 40 second run at a speed setting of 6.0 in the FastPrep® Instrument is sufficient to lyse cells, organisms and tissue present within a soil sample. If the user determines that additional processing time is required, the sample should be incubated on ice in the Lysing Matrix E tube for at least 2 minutes between successive FastPrep® Instrument homogenizations to prevent sample over-heating and possible RNA degradation.

3.5 RNA Purity, Humic Substance and Inhibitor Removal and Downstream Applications

The FastRNA® Pro Soil-Indirect Kit selectively purifies total cellular RNA (free from DNA, protein and soil components) that is sufficiently pure for use in RT-PCR and Northern analysis. While quality control tests indicate DNA removal during RNA purification, the user may incorporate DNase I treatment of the RNA prior to use in applications where absolute control of DNA contamination is required. Use DNase I at the concentration and incubation conditions recommended by the manufacturer. DNase I is inactivated by incubation at 75ºC for 5 minutes or by addition of EDTA to 25 mM followed by phenol/chloroform extraction and precipitation (14,15).

The FastRNA® Pro Soil-Indirect Kit is designed to provide two levels of RNA purification. The first level, (the basic protocol in Section 5), incorporates the proprietary RNAMATRIX® to remove soil associated reverse transcription and PCR inhibitors to allow amplification of undiluted RNA for the vast majority of soil types. It has been determined that in some instances, RNA dilution (1:3, 1:5, or 1:10) of clear or slightly colored samples may result in greater yield of PCR product. It is important to recognize that some soil samples may not RT-PCR amplify due to purification of small amounts of total or target RNA that result from a low organism content, or the soil sample may be exceptionally high in inhibiting substances, including nonspecific humic substances. If dilution of the RNA sample and nested or reamplification of the PCR reaction do not facilitate successful RT-PCR, the samples can be additionally purified using the Quick-Clean Spin Filters provided in the kit (Section 6). Centrifugation of purified RNA through the Quick-Clean Spin Filters as directed will remove residual inhibitors with no significant loss of RNA quantity.

4. Safety Precautions

RNA*pro*™ Solution contains components that when in contact with human tissue or during inhalation may cause irritation or burning. Wear personal protective equipment to prevent skin contact (e.g., gloves, lab coat, and eye protection), prevent inhalation of reagent vapors and consumption of liquid during use and dispose of waste following proper procedures. Consult the enclosed Material Safety Data Sheet for additional details.

5. Basic Protocol for All Soil Samples

1. Transfer 1 gram of soil to a 15 ml conical tube.

NOTE: Greater than 1 g of soil may be used if the soil is known to contain few microorganisms or if the RNA yield is expected to be low. Also, wet soils often provide an enriched growth environment that results in greater organism diversity and RNA yield, but since a sizeable proportion of the sample weight is due to water content, more soil may be needed. Finally, numbers of viable soil microorganisms can be increased prior to RNA isolation by incubating the soil in a liquid growth media and then proceeding directly to Step 4.

2. Add 5 ml of user-supplied Resuspension Solution (either deionized H_2O, 10 mM Tris [pH 7.0 – 8.0], or 100 mM NaCl).

NOTE: Maintain a ratio of 1 gram soil to 5 ml Resuspension Solution. Larger soil amounts may be processed in a larger tube.

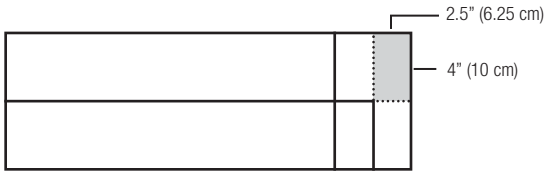
3. Mix well and incubate for 10 minutes at room temperature on a rocking device or with frequent inversion to facilitate the dissociation of organic materials from soil particles.

4. Centrifuge 5 minutes at less than 500 rpm (approximately 20 x g) at room temperature to pellet soil and large particles.

NOTE: Centrifugation is intended to remove the bulk of soil components from solution while permitting microorganisms to remain in solution. Depending on soil type, a lower speed may be used.

5. Filter the supernatant through Soil Filtration Cheesecloth to a new centrifuge tube capable of withstanding a force of 1,800 x g.

NOTE: Cheesecloth filtration is necessary in order to remove soil particles that may have carried over from the previous centrifugation. The cheesecloth is provided as a single piece with 4 layers which should be cut using scissors or a cutting board to 2.5 inches x 4 inches (6.25 centimeters x 10 centimeters) prior to use. Cutting the cheesecloth in half lengthwise provides 4 inch (10 centimeters) strips that can then be cut to 2.5 inches (6.25 centimeters) [See figure].



Fold the cut piece of cheesecloth along the 4 inch side in half to make a rectangle 2.5 by 2 inches. The folded cheesecloth will have 8 layers. Hold the folded cheesecloth taught over the tube opening or place the cheesecloth into a funnel for pouring/filtering the supernatant into the tube.

DO NOT filter through sterile gauze, Kim-Wipe™ tissue or commercially available coffee filters as SIGNIFICANT reduction in RNA yield will occur.

6. Centrifuge at 5000 rpm (approximately 1,800 x g) for 5 minutes at room temperature to pellet microorganisms and organic material for RNA purification.

7. Decant the supernatant and add 1 ml of RNA*pro*™ Solution to the tube. Completely resuspend the pelletted material by pipetting or vortexing.

8. Transfer 1 ml of the resuspended material to a Lysing Matrix E tube provided in the kit. Securely close the cap to prevent leakage in the next step.

NOTE: The designated volumes ensure adequate airspace in the matrix tube to prevent sample leakage and/or tube failure. DO NOT overfill the matrix tube. Use a second matrix tube to process a larger sample.

9. Process the sample in the FastPrep® Instrument for 40 seconds at a setting of 6.0.

10. Remove the tube and centrifuge at ≥ 14,000 x g for 5 minutes at room temperature.

11. Transfer the liquid to a microcentrifuge tube.

NOTE: Minimal debris carryover will not affect subsequent steps.

12. Incubate the sample 5 minutes at room temperature to increase RNA yield.

13. Add 300 µl of user-supplied chloroform (NO isoamyl alcohol) and vortex 10 seconds.

14. Incubate 5 minutes at room temperature to permit nucleoprotein dissociation and increase RNA purity.

15. Centrifuge at ≥ 14,000 x g for 5 minutes at 4º C.

16. Remove the upper aqueous phase to a new microcentrifuge tube without disturbing the interphase.

NOTE: If a portion of the interphase is accidentally transferred, repeat the centrifugation in step 15 with the contaminated upper phase and transfer the new upper phase to a clean microcentrifuge tube.

17. Add 200 µl of Inhibitor Removal Solution. Invert 5 times to completely mix.

18. Centrifuge at ≥ 14,000 x g for 5 minutes at room temperature.

19. Remove the liquid above the pellet to a new microcentrifuge tube.

NOTE: Following centrifugation, a 10 – 25 µl "bubble" may appear over a debris pellet. If a bubble appears, transfer only the liquid above the bubble to a new RNase-free microcentrifuge tube.

20. Add 660 µl of cold 100% isopropanol to the sample, invert 5 times to mix and place at -20ºC for at least 30 minutes. If necessary, the sample can be left overnight at this step.

NOTE: White strands may be observed in some samples. The strands, which include DNA and humic substances, will be removed in subsequent steps.

21. Centrifuge at ≥ 14,000 x g for 15 minutes at 4º C and discard the supernatant.

NOTE: The RNA pellet may appear as chocolate-colored or "dirty" due to the presence of humic substances contamination. The amount of color and contamination will vary between soil samples and will be removed in subsequent steps.

22. Carefully wash the pellet with 500 µl of cold 70% ethanol (made with DEPC-H_2O).

23. Remove the ethanol and air dry the pellet 5 minutes at room temperature.

NOTE: DO NOT completely dry the RNA as this will increase the difficulty of resuspending the RNA in the next step.

24. Resuspend the RNA in 200 µl of DEPC-H_2O.

25. Add 600 µl of RNAMATRIX® Binding Solution and 10 µl of RNAMATRIX® Slurry to the RNA. Incubate at room temperature on a shaker table, a rotator, or with frequent inversion for 5 minutes.

26. Microcentrifuge (pulse-spin) approximately 10 seconds to pellet the RNAMATRIX®-bound RNA and discard the supernatant. Use caution not to remove the RNAMATRIX®.

27. Completely resuspend the RNAMATRIX®-bound RNA in 500 µl of prepared RNAMATRIX® Wash Solution.

NOTE: Ensure that 15 ml of ethanol has been added to the RNAMATRIX® Wash Solution Concentrate prior to use.

28. Microcentrifuge (pulse-spin) approximately 10 seconds and discard the supernatant. Use caution not to remove the RNAMATRIX®.

29. Microcentrifuge (pulse-spin) a second time for approximately 10 seconds and carefully remove any residual wash solution with a pipet. Use caution not to remove the RNAMATRIX®.

30. Air dry 5 minutes at room temperature.

31. Add 50 µl of DEPC-H_2O and completely resuspend the RNAMATRIX® by vortexing. Incubate 5 minutes at room temperature to elute the RNA. Finger tap the tube bottom frequently to provide gentle mixing.

32. Microcentrifuge (pulse-spin) approximately 10 seconds and transfer the supernatant containing eluted RNA to a new tube.

NOTE: Do not discard the RNAMATRIX® pellet!

33. Repeat step 31 and 32 to provide a final RNA volume of 100 µl.

NOTE: If matrix carryover occurs, remove the matrix by pulse-centrifuging the microcentrifuge tube for approximately 10 seconds to pellet the matrix. Carefully transfer only the supernatant to a new RNase-free microcentrifuge tube.

34. Evaluate the purified RNA by RT-PCR.
If the purified RNA appears colorless it is acceptable for use in Northern analysis and should perform satisfactorily in RT-PCR. For RT-PCR amplification, it is recommended to test 1 µl undiluted and 1 µl of RNA diluted 1:3, 1:5 and 1:10. If the RNA does not amplify satisfactorily, continue with additional purification using Quick-Clean Spin Filters to remove residual inhibiting substances (Section 6).

NOTE: While it may be possible to measure the quantity and integrity of the RNA prior to RT-PCR, in most cases the sample may be too dilute for accurate detection via spectrophotometric or agarose gel analysis. Read Section 8.1 for more information.

35. Aliquot and store the RNA at -70ºC.

NOTE: RNA is generally stable for up to a year at -70 to -80ºC. For longer-term storage, RNA samples may be stored as ethanol precipitates. When stored as an ethanol precipitate, the RNA must be spun down, washed and resuspended in aqueous solution prior to use. Avoid frequent sample freeze-thaw by storing isolated RNA as aliquots.

NOTE: RNA does not evenly distribute in ethanol and can lead to inconsistent RNA amounts between samples when equal volumes are pipetted. Vortex the RNA:ethanol solution to disperse the RNA prior to removing the sample. In situations where precise amounts of RNA are required it is best to precipitate the total amount (or excess) of RNA required, resuspend the RNA in DEPC-H_2O and measure the concentration by OD_{260} before proceeding.

6. Optional Centrifugation Through Quick-Clean Spin Filters

The FastRNA® Pro Soil-Indirect Kit is designed to remove reverse transcription and PCR inhibitors to allow amplification of undiluted RNA for the vast majority of soil types. It has been determined that in some instances, RNA dilution (1:3, 1:5, or 1:10) may result in greater yield of PCR product. It is important to recognize that some soil samples may not RT-PCR amplify due to purification of small amounts of total or target RNA that result from a low organism content, or the soil sample may be exceptionally high in inhibiting substances, including nonspecific humic substances. If dilution of the RNA sample and nested or reamplification of the PCR reaction do not facilitate successful RT-PCR, the samples can be additionally purified using the Quick-Clean Spin Filters. Centrifugation of purified RNA through the Quick-Clean Spin Filter as directed will remove residual inhibitors with no significant loss of RNA quantity.

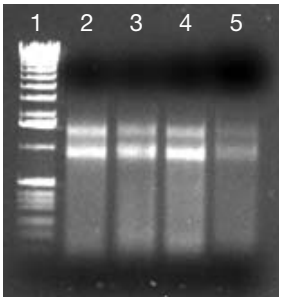
1. If the RNA sample is frozen, thaw completely and centrifuge briefly to bring the liquid to the tube bottom before proceeding.

2. Apply 50 µl DEPC-H_2O to a Quick-Clean Spin Filter, which has been inserted into a cap-less catch tube, and pulse spin for 10 seconds.
3. Transfer the Quick-Clean Spin Filter to a new, kit supplied RNase-free Catch Tube, insert into the micro-centrifuge rotor, and apply the RNA (up to 300 ml may be processed) to the Quick-Clean Spin Filter.

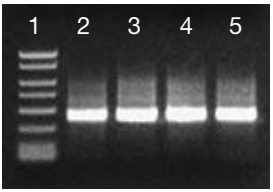
NOTE: Do not leave the RNA in contact with the Quick-Clean Spin Filter for more than 60 seconds before pulse spinning for 10 seconds in the next step or RNA loss will occur.

4. Pulse-spin the Quick-Clean Spin Filter and Catch Tube for 10 seconds to collect purified RNA.

7. Example Data: Total RNA Isolation and RT-PCR



Total RNA extracted from Soil Samples with the FastRNA® Pro Soil-Indirect Kit. Approximately 15% of the total RNA isolated from four different soil samples was loaded on to a 0.8% agarose gel. Lane 1: 1kb ladder, Lane 2: RNA from 5 g Soil #1, Lane 3: RNA from 10 g Soil #2, Lane 4: RNA from 5 g Soil #7, Lane 5: RNA from 5 g Soil #10.



RT-PCR of Fungal Gene from Total RNA Isolated from Soil Samples with the FastRNA® Pro Soil-Indirect Kit. Approximately 40% of the RT-PCR reaction was loaded on to a 0.8% agarose gel. Lane 1: 150bp – 2kb marker, Lane 2: Soil #1, Lane 3: Soil #2, Lane 4: Soil #7, Lane 5: Soil #10.

8. Troubleshooting

8.1 Lower Than Expected or No RNA Yields

Due to natural soil diversity soil samples may contain very low amounts of the desired organism(s) for extracting RNA. Additional numbers of the same sample may be processed using multiple tubes and the purified RNA pooled. Alternatively, a greater amount of soil can be used in step 1 of the protocol in section 5.

Soil samples stored for extended periods may result in organism and RNA deterioration. To prevent sample deterioration, process the sample immediately following collection. In order to understand storage deterioration in specific soil types a control stability experiment using a laboratory microorganism (i.e., *E. coli* or *S. cereviseae*) stored in the soil sample may be performed. Add equivalent amounts of microorganism to aliquots of the soil and store using the standard method. Prepare RNA from the stability samples over extended time periods (e.g., hours, days, weeks) to provide information about the relative RNA yields and losses that can be expected during storage. Aliquots of the control microorganism may also be stored without soil and processed in parallel to compare RNA yield with the soil stability samples Lack of RNA degradation in the non-soil control tube indicates the soil stability sample RNA was likely degraded during soil storage prior to the addition of RNA*pro*™ Solution.

If desired, spectrophotometric analysis can be performed following this example:

a. Dilute 5 µl of the purified RNA into 495 µl of DEPC-H_2O
b. Read the OD_{260} using DEPC-H_2O as a blank
c. Calculate the sample µg RNA per ml using the formula:
   (OD_{260})(40 µg/ml/per OD)(100 [dilution factor]) = µg RNA per ml

Spectrophotometer accuracy is greatest between ~ 0.2 and ~ 0.8. If the OD reading is below this range, add more RNA sample (e.g., 20 µl RNA + 480 µl DEPC-H_2O) or concentrate the RNA by precipitation and resuspension into a smaller volume. If the OD reading is above the recommended spectrophotometer range, use less RNA for the OD determination. If RNA yield is low, greater accuracy will be achieved by concentrating the RNA sample before analysis or use agarose gel electrophoresis to approximate the concentration.

RNA integrity and an estimation of yield can be determined by analyzing a portion of the RNA sample using gel electrophoresis and comparing it to a known amount of RNA. Take a 15 µl aliquot of RNA, add gel loading buffer (see Related Products) and load the sample and the known amount of RNA on a 1.0% agarose gel at ~ 100 volts for 30 minutes. Ethidium bromide may be added to the denatured RNA sample at 10 µg per milliliter prior to gel loading or the gel may be ethidium bromide stained and destained following electrophoresis and visualized under UV light. The quality of the RNA is determined by the appearance of distinct large and small ribosomal RNAs of approximately 0.9 to 1.5 kb. Due to the potential organism heterogeneity in a soil sample multiple bands may be present. The purified rRNA concentration may appear low, but is not completely indicative of the amount of mRNA present in the sample. RT-PCR will often yield positive results in the absence of visible rRNA.

Certain bacterial strains may contain elevated RNase levels. Reduce the exposure time to RNase by adding the RNA*pro*™ Solution to each sample as soon as possible following sample harvest. RNA*pro*™ Solution will protect RNA in soil samples from degradation for at least 24 hours at room temperature or 4°C.

8.2 Suspected RNA Degradation

The quality of RNA can be determined after electrophoresis by the appearance of distinct large and small ribosomal RNAs (rRNA) of approximately 0.9 to 1.5 kb. Due to the potential organism heterogeneity in a soil sample multiple bands may be present. The purified rRNA concentration may appear low, but is not completely indicative of the amount of mRNA present in the sample. RT-PCR will often yield positive results in the absence of visible rRNA.

Messenger RNA (mRNA), which typically represents approximately 1% of the total cellular RNA and is heterogeneous length, may not be highly visible. Ribosomal RNA is used as a marker to assess sample degradation. Degraded RNA or mRNA may appear as unequal fluorescent intensity between bands, a single rRNA band may be completely lacking or a heterogeneous fluorescent smear may appear below the rRNA bands.

The rRNA content is not an accurate indication of mRNA content purified from soil. Samples that lack visible rRNA in agarose gel electrophoresis will often function successfully in RT-PCR amplification.

RNase may have been introduced during isolation. To prevent RNAse contamination, the use of gloves, RNase-free plugged pipette tips and RNase free tubes is strongly recommended. Clean pipetmen and work area with RNase Erase® (Qbiogene Catalog # 2440-204) prior to beginning RNA isolation. Use DEPC-treated reagents. RNA*pro*™ Solution contains RNase inactivating components and will not support RNase contamination.

Ensure that DEPC-treated H_2O was used to make the 70% ethanol.

Artifactual RNA degradation may occasionally occur during gel electrophoresis due to a gel that was not RNase free, running the gel at too high voltage or from using depleted running buffer. Rerun the samples with a known intact RNA sample using freshly prepared RNase-free electrophoresis reagents.

8.3 Properties of the RNA Pellet

Following RNA precipitation the purified RNA may not appear as a pellet at the tube bottom but may instead adhere to the side of the tube. The RNA may not be visible in the pellet or on the tube side and it may appear that RNA has not been purified. Complete the protocol and confirm the RNA concentration by OD_{260} and integrity by gel electrophoresis. RNA adhering to the tube wall will not affect its purity, size or use in subsequent applications.

Following RNA precipitation the RNA pellet may not be firmly attached to the side or bottom of the tube and may be observed floating in the solution or at the solution surface. Re-centrifuge the sample in the same tube and exercise caution to not lose the pellet when removing the supernatant.

A brown color present in the RNA pellet after Step 21 is most likely due to co-purification of humic substances which will be removed by the RNAMATRIX® in steps 25-33. It has been determined that in some instances, RNA dilution (1:3, 1:5, or 1:10) may result in greater yield of PCR product. It is important to recognize that some soil samples may not RT-PCR amplify due to purification of small amounts of total or target RNA that result from a low organism content, or the soil sample may be exceptionally high in inhibiting substances, including nonspecific humic substances. If dilution of the RNA sample and nested or reamplification of the PCR reaction do not facilitate successful RT-PCR, it is recommended the samples receive additional purification using the kit provided Quick-Clean Spin Filters.

8.4 Genomic DNA Contamination

The FastRNA® Pro Soil-Indirect Kit is designed to remove genomic DNA during sample processing.

However, if genomic DNA contamination is suspected, it will appear as a high molecular weight smear on a denaturing gel. Genomic DNA contamination and/or protein contamination may appear during agarose gel electrophoresis as ethidium bromide stained material in the gel loading well. Lithium chloride (LiCl) precipitation of RNA may be used as a means to remove DNA and/or protein. Historically, LiCl has been used to precipitate RNA greater than ~300 nucleotides from tRNA and 5s RNA. LiCl precipitation may be incorporated into the FastRNA® Pro Soil-Indirect Kit procedure: To the final 100 µl RNA suspension (after step 33 of Basic Protocol for All Soil Samples), add LiCl to a final concentration of 1.6 M (e.g., 0.2 volumes or 20 µl RNase-free 8 M LiCl). Add 2.5 volumes or 250 µl RNase-free absolute ethanol. Mix and place on ice for at least 2 hours. Centrifuge for 15 minutes at >12,000 rpm at 4°C. Remove the supernatant and wash the pellet with 75% cold RNase-free ethanol. The ethanol wash step is critical to prevent LiCl inhibition of cell-free translation and in vitro transcription. Air dry the pellet. Resuspend RNA in 100 µl DEPC-H_2O.

8.5 RT-PCR Inhibition

The FastRNA® Pro Soil-Indirect Kit is designed to provide levels of RNA purification that permit tailoring the protocol to the soil sample and the amount of reverse transcription and PCR inhibitors present in the soil sample. Steps 1 – 33 provide the first level of purification using proprietary RNAMATRIX®. Section 6 provides second level purification through Quick-Clean Spin Filters. RNAMATRIX® purification provides sufficient RNA purification for the majority of soil samples that permits RNA use in Northern analysis and RT-PCR amplification without additional purification. It has been demonstrated that in some instances, RNA dilution prior to amplification (1:3, 1:5, or 1:10) may result in greater yield of PCR product. It is important to recognize that individual soil samples that are resistant to RT-PCR may have a low amount of RNA due to low organism content or may be so high in inhibiting substances, including nonspecific humic acids, that dilution of the RNA sample still does not allow successful RT-PCR. For these samples, Qbiogene has included Quick-Clean Spin Filters in the FastRNA® Pro Soil-Indirect Kit as an optional last step. Centrifugation of purified RNA through the Quick-Clean Spin Filter as directed will remove any residual enzymatic inhibitors with no significant loss of RNA quantity.

Unsuccessful RT-PCR may also result from the inadvertent introduction of RNase into RT-PCR reagents during experimental handling. Include a control RNA with the RT-PCR reagents to test for RNA degradation.

Unsuccessful RT-PCR may result if the reverse transcriptase and/or the thermostable polymerase is inactive or was not added to the reaction, or if other solutions are compromised or omitted. Perform RT-PCR using enzymes and buffers with a known control RNA and primers.

Unsuccessful RT-PCR may also result if PCR primer conditions have not been optimized. Test the amplification primers using a control RNA to confirm the ideal annealing temperature and concentration.

8.6 Mucopolysaccharide / Carbohydrate Contamination

Cellular mucopolysaccharides will not co-purify with RNA using the protocol and reagents in the FastRNA® Pro Soil-Indirect Kit.

9. Recommended Reference Format for Publication

Total RNA was isolated from _____ g of (type) soil using the FastRNA® Pro Soil-Indirect Kit (Qbiogene, Inc., CA) and FastPrep® Instrument (Qbiogene, Inc, CA), for _____ seconds at a speed setting of _____.

10. References

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11. Related Products

11.1 FastRNA® Pro Soil-Direct Kit (Cat# 6070-050)

The FastRNA® Pro Soil-Direct Kit is designed to efficiently isolate total RNA from organic material found in soil samples. Soil is homogenized by the FastPrep® Instrument in impact-resistant 2 ml tubes containing Lysing Matrix E. Total RNA is released into a protective solution, extracted, precipitated, and purified from inhibiting substances with the proprietary RNAMATRIX® and optional Quick-Clean Spin Filter. Total RNA prepared with this kit is suitable for RT-PCR and other applications.

11.2 Other Related Products

Item	Size/Description	Catalog #
FastPrep® FP100A Instrument	100V	6001-100
FastPrep® FP120A Instrument	120V	6001-120
FastPrep® FP220A Instrument	220V	6001-220
FastRNA® Pro Soil-Direct Kit	50 preps	6070-050
FastRNA® Pro Red Kit (Yeast)	50 preps	6035-050
FastRNA® Pro Green Kit (Plant & Animal)	50 preps	6045-050
FastRNA® Pro Blue Kit (Bacteria)	50 preps	6025-050
FastDNA® Kit	100 preps	6540-400
FastDNA® SPIN Kit for Soil	50 preps	6560-200
FastProtein™ Blue Matrix	50 preps	6550-400
FastProtein™ Red Matrix	50 preps	6550-600
RNase Erase®	500 ml	2440-204
QA-Agarose, Molecular Biology Grade	500 g	AGAH0500
BGFNE (alkaline agarose gel loading dye)	1 ml	2339-104
BBXFE (denaturing RNA gel loading dye)	1 ml	2343-104
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